SEVEN-MONTH PROGRESS REPORT

CTR GRANT #629C

PROGRESS REPORT NO. 1

Carlton K. Erickson, Ph.D.
School of Pharmacy
Department of Pharmacology and Toxicology
The University of Kansas
Lawrence, Kansas

June 1, 1972 - Dec. 31, 1972

Effects of Nicotine on Free Acetylcholine in the Hippocampus During Learning

The application for a research grant submitted to CTR-USA on February 7, 1972 for this new project listed two major objectives (with several associated techniques) as follows:

1. To measure the output of acetylcholine (ACh) from the hippocampus (HPC) in restrained rabbits, before and after the administration of learning-enhancement doses of nicotine.
   a. Perfusion of the HPC with "push-pull cannulas".
   b. Recording of EEG from the HPC with bipolar electrodes contralateral to the cannula.
   c. Measurement of blood pressure for monitoring the cardiovascular effects of nicotine.
   d. Refinement of the leech muscle ACh bioassay.
2. To do similar experiments in rats that are actively learning a conditioned avoidance response.
   a. Development of small push-pull cannulas to perfuse the HPC of rats.
   b. Development of leech-muscle assay sensitivity to measure rat-brain quantities of ACh.
   c. Perfusion of HPC during behavioral performance (i.e. avoidance learning) in rats.
   d. Correlation of HPC ACh output with nicotine-enhanced avoidance learning.

During the first half of the project, we have collected a very large amount of valuable data. Not only is the above outline about 5/8 completed, but other related behavioral and telemetry experiments with nicotine have proven extremely fruitful. The reasons for this success are many: a) the experiments have run smoothly because most of the techniques had already been developed for another project, b) two undergraduate students were available to help with collection of data, and c) most experiments gave positive results without the procedural problems which had impeded us in our last nicotine projects.

Results of experiments which have been completed or are in progress are as follows:

1. Analysis of free ACh in the rabbit HPC (almost complete) - A total of 20 rabbits was used to obtain HPC perfusates before and after

1003538851

Source: https://www.tobaccodocuments.org/ctr/00000000

nicotine. Contralateral EEG and femoral artery blood pressure were monitored with a Grass Model 7 polygraph. Nicotine was given in intravenous doses of 0.2, 0.4, and 0.6 mg/kg, which are roughly equivalent to intraperitoneal avoidance-facilitating doses in rats. The time-sequence effects of each dose of nicotine on ACh levels in 15-minute perfusate samples from the HPC are shown in Figures 1, 2, and 3. As can be seen, 0.2 mg/kg nicotine had very little effect on the release of ACh from the HPC, 0.4 mg/kg had a significant effect, and 0.6 mg/kg had a large effect. Furthermore, when a second dose (0.4 mg/kg) of nicotine followed the initial dose (Figure 2), there was an even greater effect on ACh release, perhaps due to cumulative action of the two doses. Interestingly, the effects of the second dose were more fleeting than the effects of the first dose, a suggestion that ACh stores were being depleted by nicotine. The implications of such observations are that nicotine is apparently releasing "free" ACh in the HPC at a rate faster than it can be synthesized, and this effect, if it is occurring in the rat during nicotine-enhanced avoidance learning, means that the excess free ACh could be enhancing the activity of the HPC to cause a direct increase in learning speed. It is probable that there is an optimal level of ACh required for enhanced learning, and that levels that are too high (or too low, if ACh becomes too depleted) would not affect learning. Data aimed at looking further into this mechanism will be collected in the rat experiments.

Figure 4 shows the true dose response nature of nicotine's effect on free ACh. Figure 5 shows that these doses produce theta rhythm, which is characteristic of cholinergic drug effects in the HPC, in some rabbits. Blood pressure from these doses of nicotine is not greatly affected, although in some cases the drug may produce a slight depression of blood pressure. Figures 6 and 7 are cross-sections of the rabbit brain showing the position of the cannula and electrode.

2. Analysis of free ACh in the rat HPC during learning (over ½ complete) - Push-pull cannulas have been obtained from Plastic Products Company (Roanoke, Virginia) and have been unilaterally implanted into the HPC of approximately 15 rats. Perfusates have been collected intermittently, with the major problem being clotting of the "pull" side of the cannula (with a subsequent increase in intracranial pressure and death). When perfusates have been successfully collected, they have not been assayed, since the animals up to this point have been anesthetized, and it is known that anesthetics reduce ACh levels to almost imperceptible levels. The important finding, however, is that the quantities of perfusate appear to be great enough to be measured by the leech bioassay technique. A few trials have been run in which blocked cannulas were used while the rat was awake, to find out how the two polyethylene tubes would react to constant turning and twisting by the animal. It appears that this will not present much of a problem. We are continuing to work on the clotting problem of the push-pull cannula, having consulted with Dr. Larry Stein of Wyeth Laboratories, who has published on this technique.

In addition to the above major portions of the proposed project, the following related studies have been done which are extensions of the project funded by CTR-USA last year:

3. Blockade by mecamylamine of nicotine-enhanced avoidance behavior -

10035388852

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

Mecamylamine, a proposed central nicotine antagonist, was injected intraperitoneally into rats 20 minutes prior to nicotine, which was given immediately before every daily one-hour session during discriminated leverpress avoidance training. As can be seen from Figure 8, the larger (1.0 mg/kg) of two doses of mecamylamine completely blocked the avoidance-enhancing effects of nicotine, whereas the smaller dose (0.5 mg/kg) had no effect. Appropriate controls show that the large dose of mecamylamine alone did not affect avoidance behavior compared with saline control. Although the exact central mechanism of mecamylamine is unknown, we have confirmed earlier studies by other workers who showed that mecamylamine does antagonize the central effects of nicotine.

4. Effects of avoidance-enhancing doses of nicotine on telemetry-monitored HPC EEG - In an attempt to correlate nicotine-learning facilitation with changes in HPC EEG, unilateral bipolar stainless steel or platinum electrodes were implanted in the HPC of about 20 rats. A back-pack transmitter was connected to the electrodes when each animal was tested, and the signal sent out by the transmitter was received by a Narco Biosystems Telemetry Receiver, the output of which was amplified and recorded on a Narco Biosystems 4-channel physiograph. Figure 9 shows a typical record of HPC EEG from an awake, moving rat. Although "movement artifacts" were a problem at times, sufficiently interference-free recordings were made to allow EEG monitoring after doses of 0.4-0.6 mg/kg nicotine intraperitoneally. No significant changes in HPC EEG were seen after nicotine. Although previous work in our laboratories has shown that arecoline, a strong cholinergic stimulant, would produce "theta rhythm" (4-7 H_z recordings) in rats with wires connecting the HPC electrodes and the recorder, similar doses of arecoline did not produce theta rhythm in the telemetry-monitored preparation. We are continuing to study the reasons for this by returning to acute, anesthetized rat studies in an attempt to record arecoline-induced theta rhythm with telemetry. After we do this experiment, we will have a better idea about what our problems are.

5. Lack of effect of atropine in blockade of nicotine-enhanced avoidance behavior - In an attempt to replicate and expand upon our earlier work which showed that atropine, a specific "muscarinic" cholinergic blocker, could prevent nicotine's effect on avoidance learning, we again injected 1.0 mg/kg atropine intraperitoneally into rats 20 minutes before nicotine was given at the start of each daily one-hour training session. Providing appropriate controls (saline, atropine alone), and trying three 15-session replications, we were unable to show, as we have before, that atropine had any blocking effect (see Figure 10). There are a number of possible explanations for this, including seasonal variation, different ages of animals, inability to pick out atropine's effect within each hour session (whereas earlier atropine had a longer duration and affected the whole hour's avoidance), animal supplier variation, etc. We would like to continue to look at this problem, using a large group of new rats, all from the same supplier within a short time span, and with behavioral equipment that will allow us to break down the behavior of the animals within each hour. The answer to this problem may provide an important clue to nicotine's central mechanism.

1003538853

Source: https://www.industrydocuments.ucsf.edu/docs/

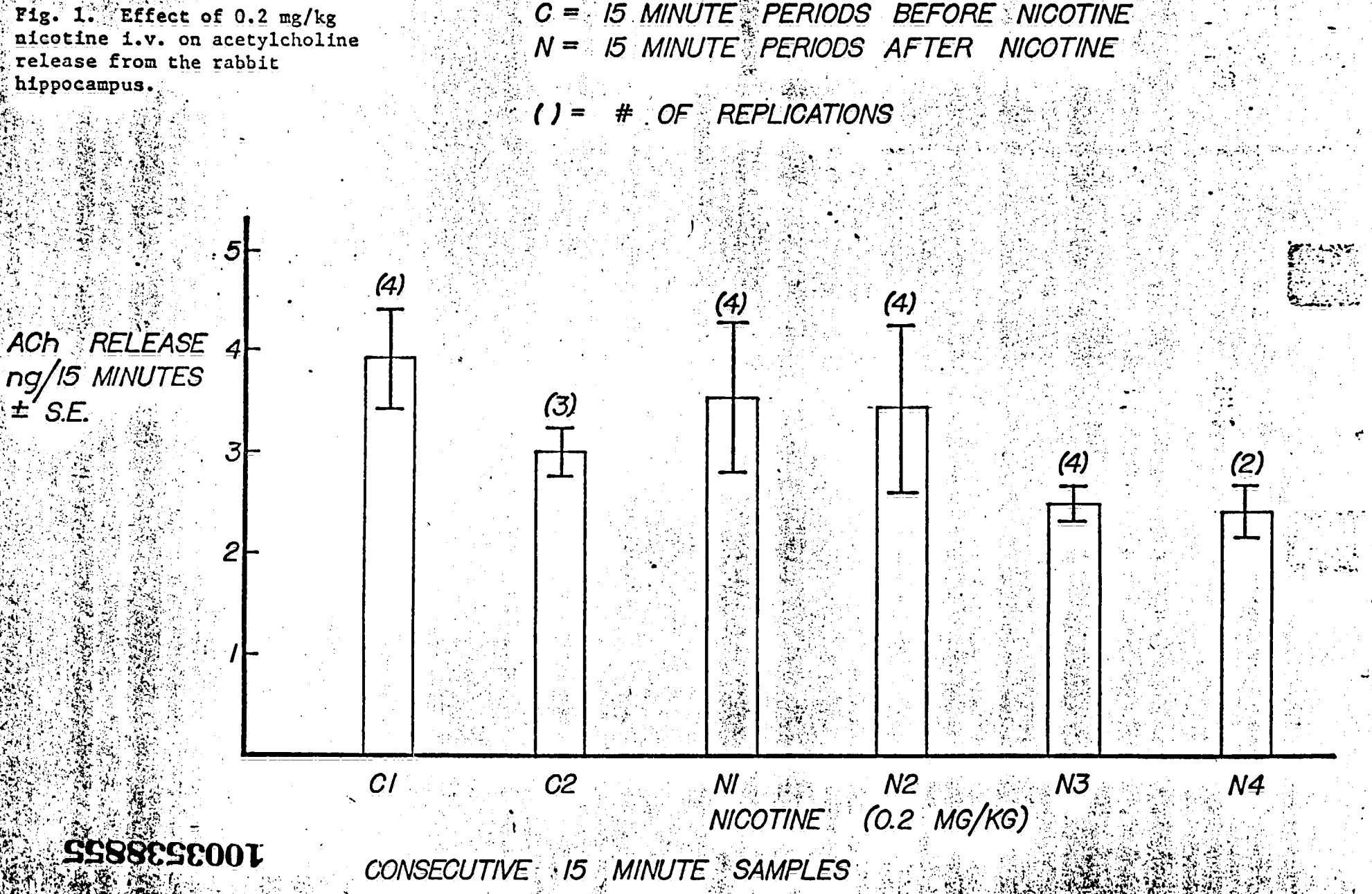
Summary

A large quantity of valuable information has been collected during the first 7 months of the study. Of greatest importance is the discovery that nicotine causes the release of free ACh from the HPC in a dose-response manner, thereby proving part of the proposed hypothesis. Other important information is the discovery that mecamylamine, a central nicotine antagonist, can prevent the learning-enhancing effects of nicotine. Of potential importance is the finding that atropine, unlike its effects in our earlier work, will not antagonize nicotine-learning enhancement at this time. The reasons for this must be determined. Finally, important developmental work has been done on a) the methods of telemetry-monitored HPC EEG and push-pull cannula perfusion of the HPC in freely-moving, awake rats, and b) their application to the study of nicotine's effects on learning.

1003538854

Source: https://www.industrydocuments.ucsf.edu/docs/xxxx0000

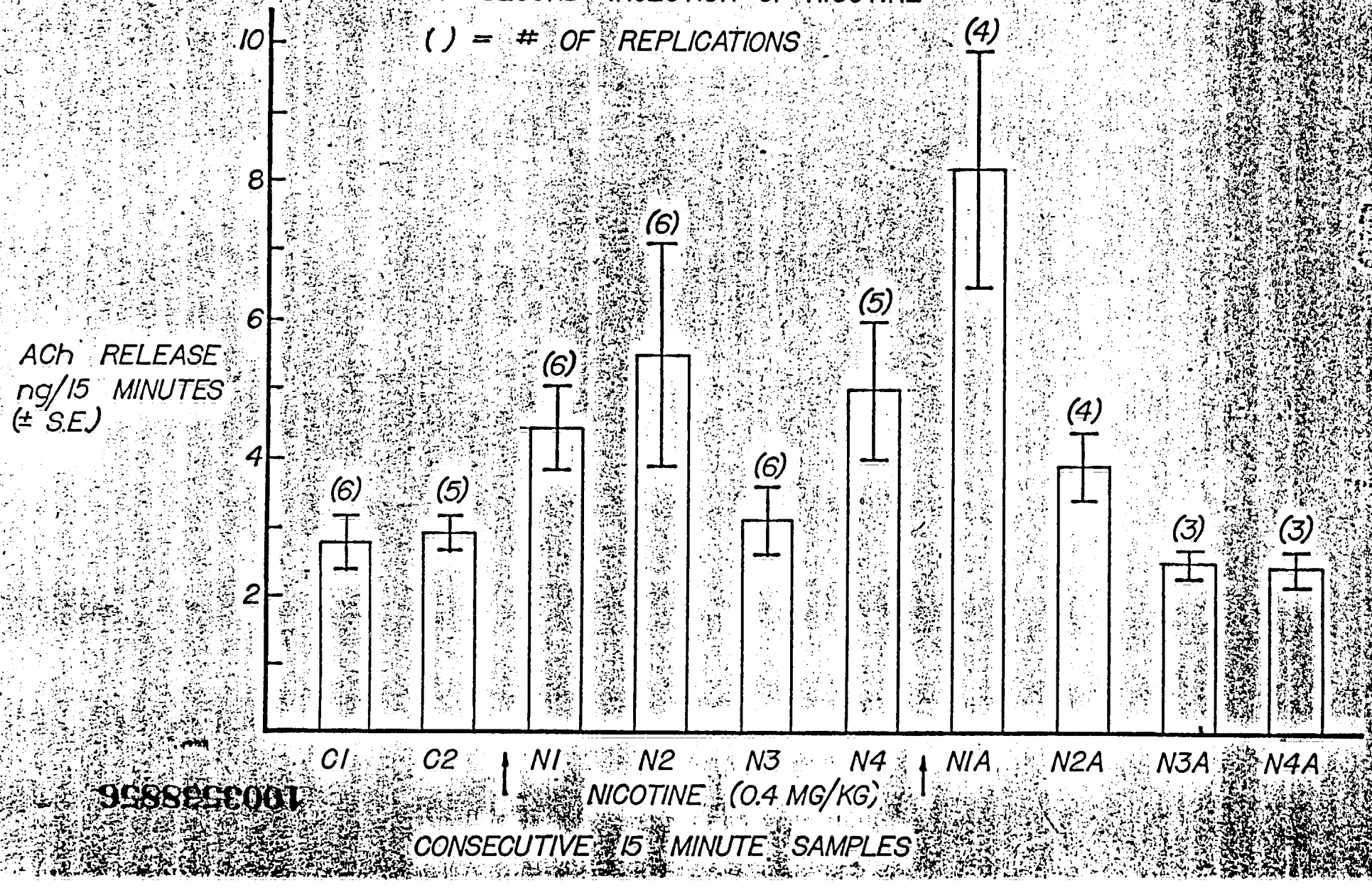
Fig. 1. Effect of 0.2 mg/kg nicotine i.v. on acetylcholine release from the rabbit hippocampus.



1003538855

Source: https://www.industrydocuments.ucsf.edu/docs/sryl0000

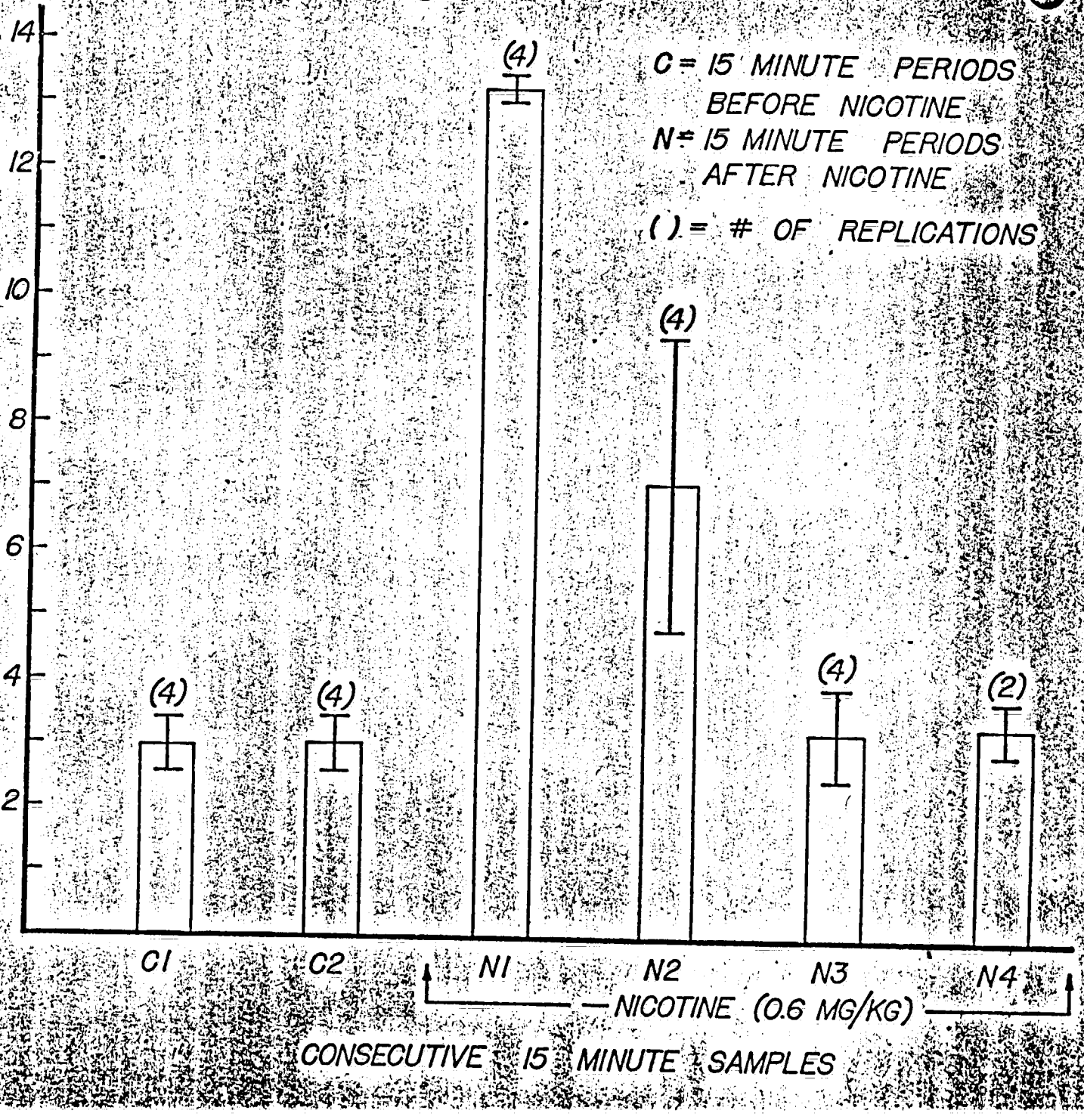
Fig. 2. Effect of 0.4 mg/kg nicotine i.v. on acetylcholine release from the rabbit hippocampus. The same dose of nicotine was given again at the second arrow.



1003388356

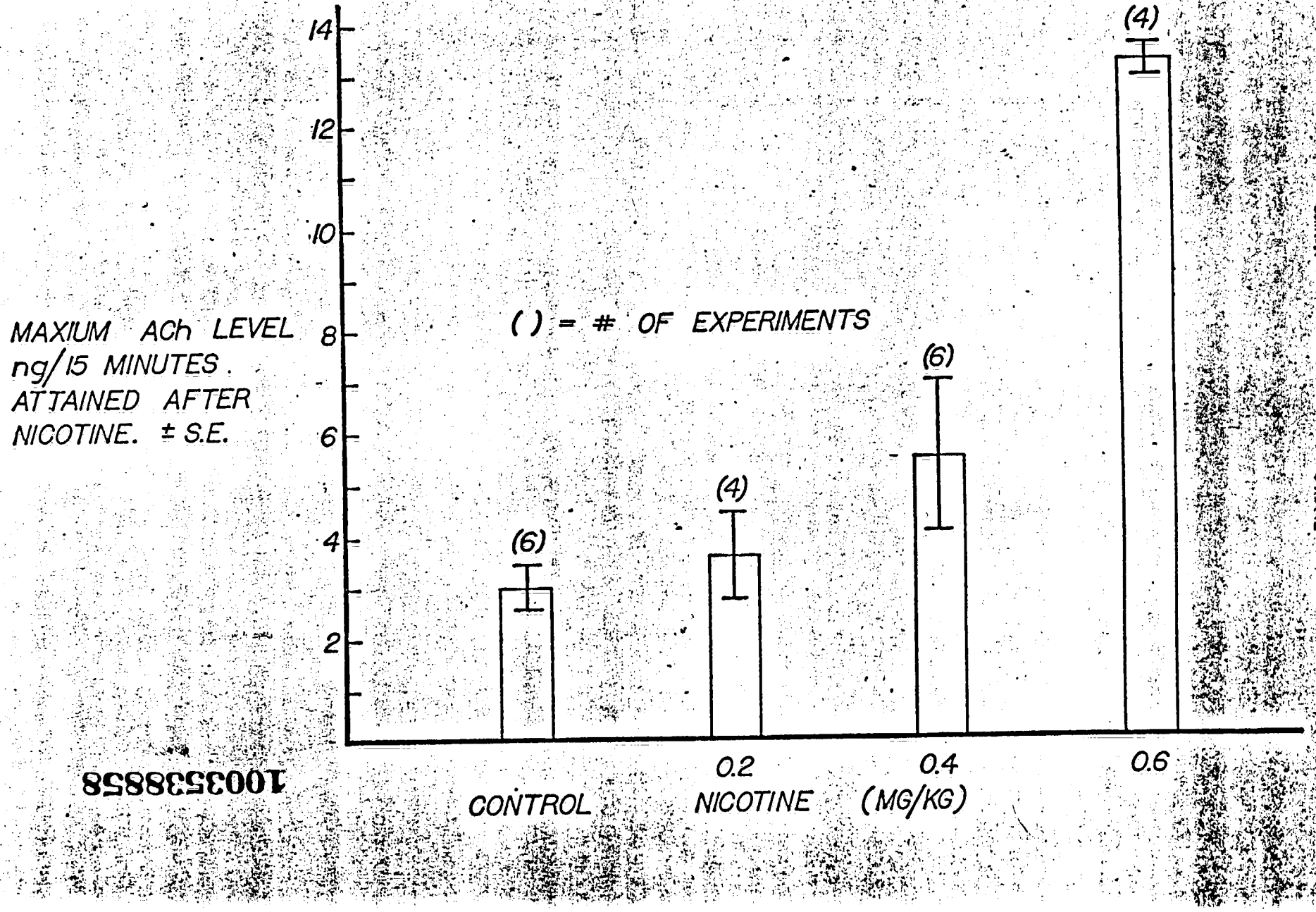
Source: https://www.industrydocuments.ucsf.edu/docs/sryf0000

Fig. 3. Effect of 0.6 mg/kg nicotine i.v. on acetylcholine release from the rabbit hippocampus.



Source: https://www.industrydocuments.ucsf.edu/docs/sryf0000

Fig. 4. Maximum acetylcholine release from the rabbit hippocampus following the three doses of nicotine, and during control (no nicotine) determinations.



1003388258

Source: https://www.industrydocuments.ucsf.edu/docs/sryk0000

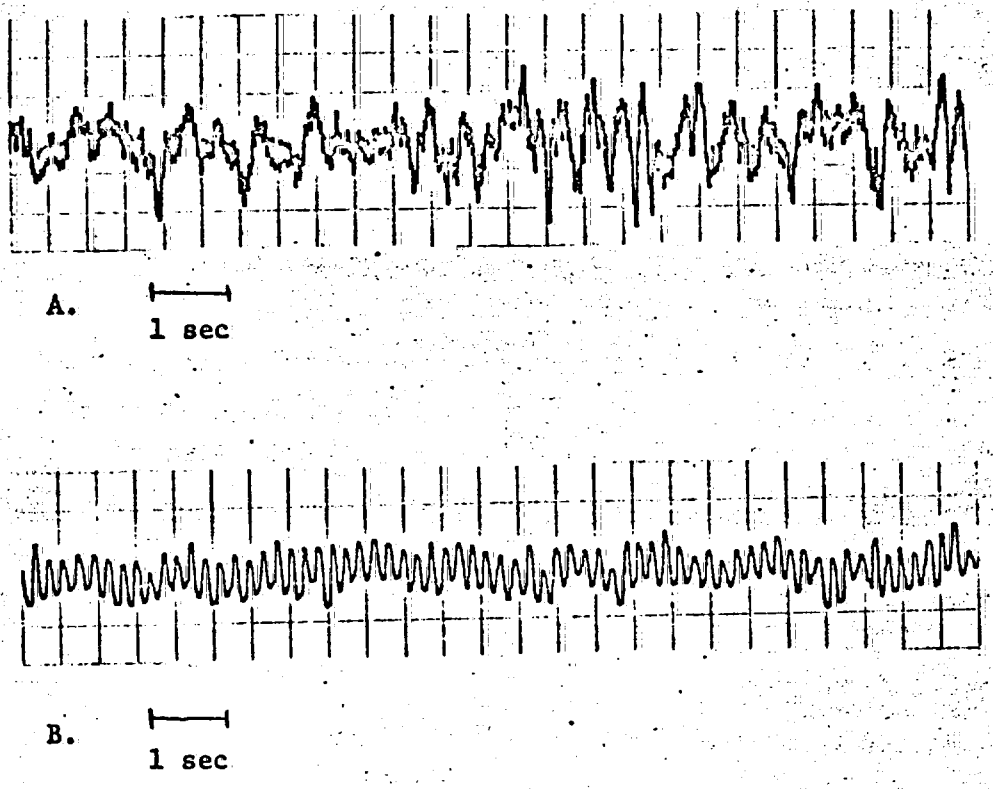


Fig. 5. Representative hippocampal electrical patterns in an unanesthetized, immobilized (gallamine) rabbit before (A) and 20 minutes after (B) an intravenous dose of 0.4 mg/kg nicotine. Calibration sensitivity for both records is the same. Activity in B is theta rhythm, 4-7 H_2.

1003538859

Source: https://www.industrydocuments.ucsf.edu/docs/syfb0000

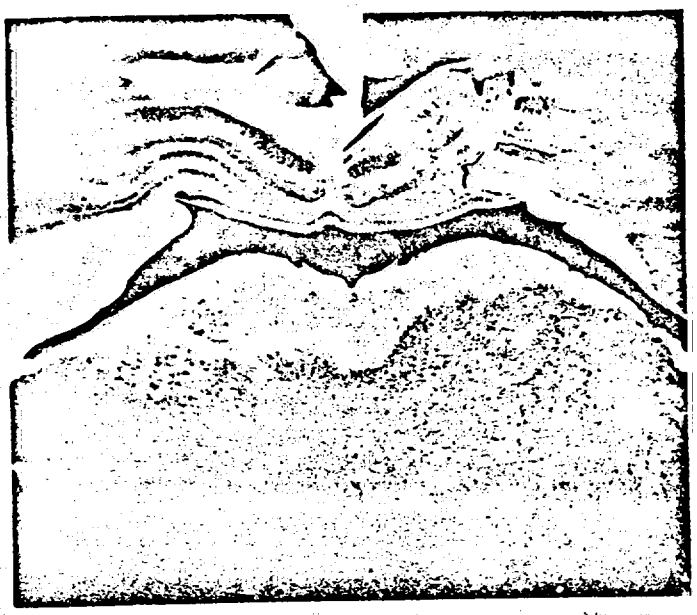


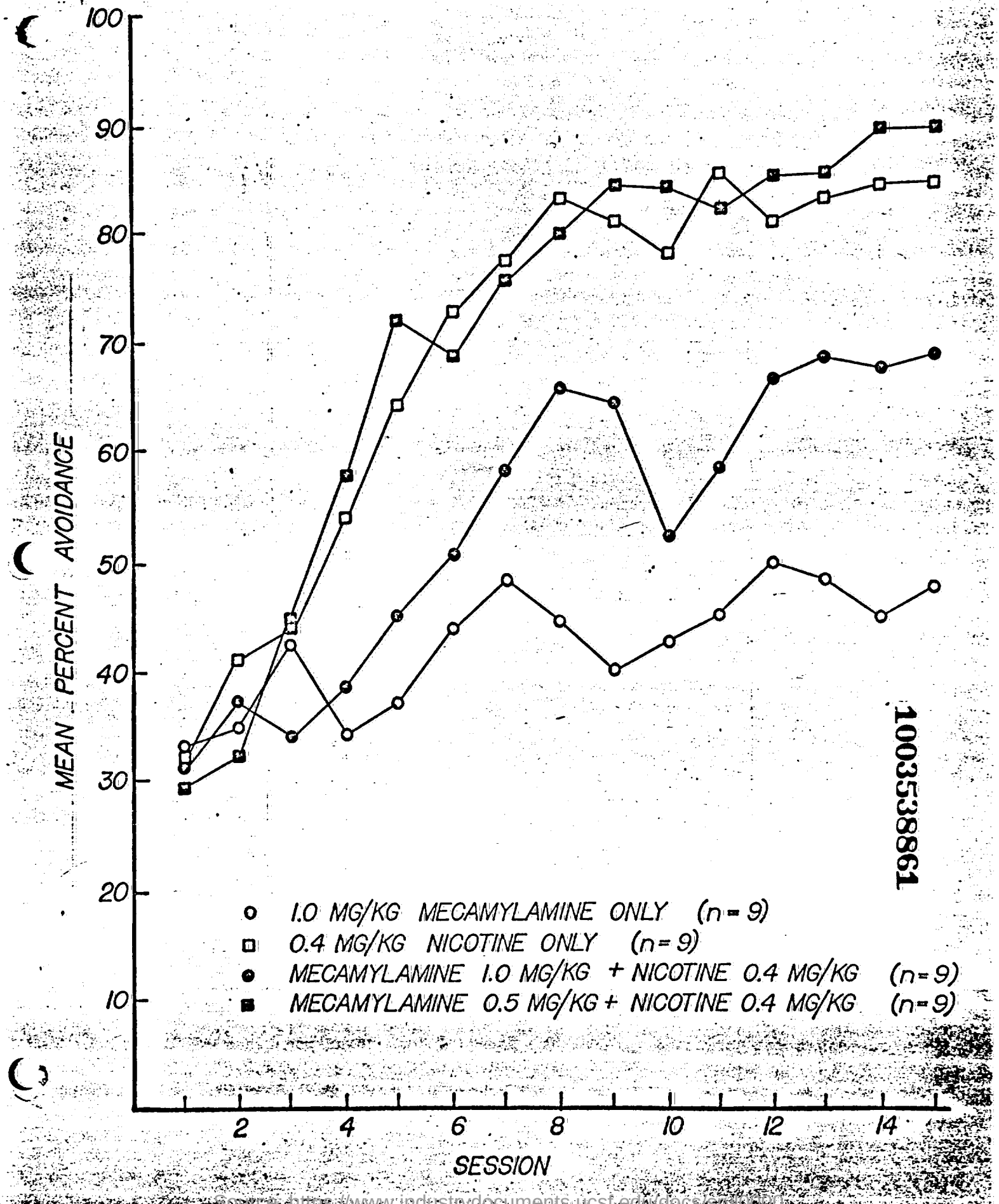
Fig. 6. Cross section of rabbit brain, showing push-pull cannula placement in the dorsal hippocampus.

Fig. 7. Cross section of rabbit brain, showing bipolar electrode placement in dorsal hippocampus, contralateral to and approximately 0.5 mm rostral to cannula tract in Fig. 6.

100353886O

Source: https://www.industrydocuments.ucsf.edu/docs/zqf00d

Fig. 8. Blockade of nicotine-induced conditioned avoidance learning enhancement by two doses of mecamylamine given intraperitoneally.



Source: https://www.industrydocuments.ucsf.edu/docs/

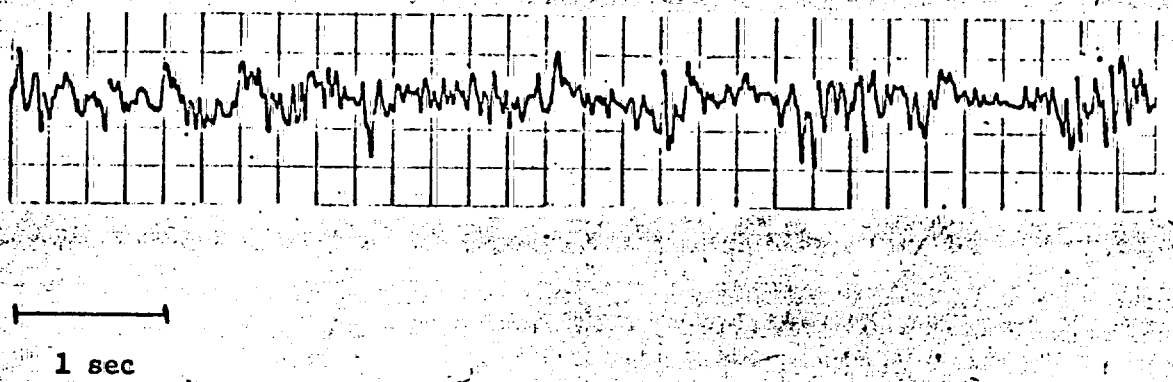
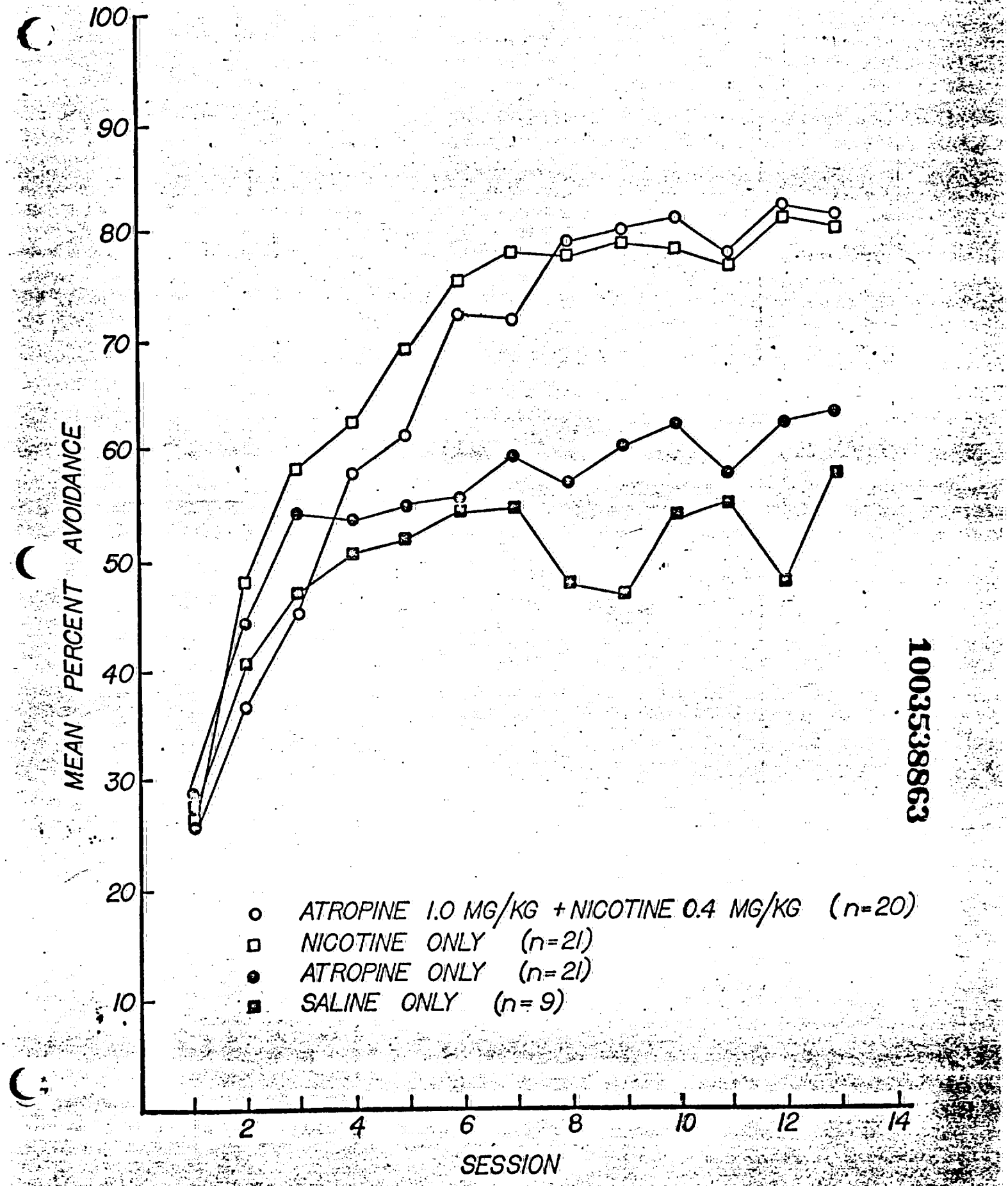


Fig. 9. Representative hippocampal electrical activity through platinum electrodes via telemetry in a rat (uncalibrated amplitude).

100353886

Source: https://www.industrydocuments.ucsf.edu/docs/[illegible]

Fig. 10. Lack of blockade of nicotine-induced conditioned avoidance learning enhancement by atropine given intraperitoneally.



100538863

Source: https://www.industrydocuments.ucsf.edu/docs/